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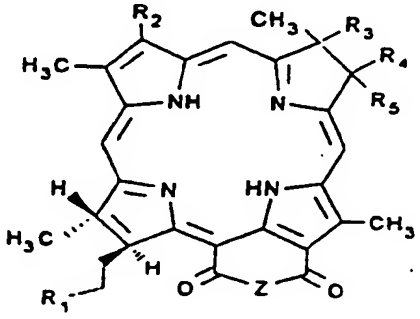
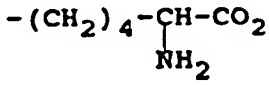
(54) Title: PURPURIN-18 ANHYDRIDES AND IMIDES AS PHOTOSENSITIZERS

(57) Abstract

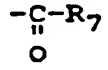
Compounds having utility as light absorbing compounds, especially in the area of photodynamic therapy. Such compounds have formula (I) where z is =0 or =NR_6, where R_6 is lower alkyl of from 1 to 8 carbon atoms or (a); R_1 is (b), where R_7 is (c) or -OR_8, where R_8 is hydrogen or lower alkyl of 1 to 8 carbon atoms, or R_7 is an amino acid residue connected at a nitrogen atom of such residue; R_2 is lower alkyl or lower alkylene of from 2 to 4 carbon atoms or a f rmal r carbonyl containing group of from 1 to 4 carbon atoms; R_3 and R_4 are -H or -OR_8 or are joined together so that each represents one-half of a chemical bond and R_4 may be taken together with R_5 to form =0; and R_5 is ethyl or is taken together with R_4 to form =0; provided that when Z is -0- the sum of the number of carbon atoms in R_1 through R_5 is from 12 to 20 and when Z is -NR_6, the sum of the number of carbon atoms in R_1 through R_6 is 8 to 20.



(I)



(a)



(b)

PURPURIN-18 ANHYDRIDES AND IMIDES AS PHOTOSENSITIZERS

This work was supported by grants from the National Institute of Health (CA 52997, CA 55791, HL 22252) and Oncologic Foundation of Buffalo, New York. The United States Government may have certain rights in the invention.

Background of the Invention

Photosensitizers are chemicals which kill cells and/or fluoresce when activated by light of a specific wavelength. Most malignant and some premalignant tissues retain these photochemically active substances in higher concentrations and for longer durations than surrounding normal tissues. The retention time is not dependent on wether or not the cells are synthesizing DNA or cell growth or nutrient uptake. This form of treatment, therefore, is an important new part of cancer treatment and tumor detection (Dougherty, T.J., *CRC Critical Rev. Oncol. Hematol.*, 1984, 83).

Photosensitizers have been recognized for almost a century. In 1900, (Rabb, C., *Z. Biol.*, 1900, 39, 1423) reported the lethal effects of a combination of acridin orange dye and ordinary light on *Paramecium*. In 1903, von Tappeneir reported the first therapeutic use of photosensitizers when he used eosin and white light to treat skin tumors. The phototoxic effect of an administered porphyrin in man was observed in 1913. The localization of administered porphyrins in tumor tissue was recognized in the 1940s. It was not until 1972, however, that these two ideas (photodegradation of tissue and localization in tumors) came together successfully, when Diamond demonstrated that a porphyrin could preferentially degrade tumor implants in a rat (Diamond, I.; McDonagh, A.F.; Wilson, C.B.; Granelli, S.G.; Nielsen, S.; Jaenicke, R., Lancet, 1972, 1175). This result was confirmed and extended by Dougherty, T.J.; Grindey, G.B.; Fiel, R.; Weishaput, K.R.; Boyle, D.G.; *J. Natl. Cancer Inst.*, 1975, 55, 115.

derivative (Hpd) is prepared by mixing hematoporphyrin with glacial acetic acid and sulfuric acid, followed by hydrolysis and precipitation under acidic conditions. This method was partially described by Lipson et al (Lipson, R.L.; Baldes, E.J.; Olsen, A.M., *J. Natl. Cancer Inst.*, 1961, 26, 1). Hpd thus produced consists of a variety of porphyrins. When Hpd is separated into its two main fractions by gel filtration with Sephadex LH-20, the higher molecular weight portion, called Photofrin®, is a more efficient PDT agent (Dougherty, T.J.; Boyle, D.G.; Weishaupt, K.R.; Henderson, B.; Potter, W.; Bellnier, D.A.; Wityk, K.E., *Adv. Exp. Biol. Med.*, 1983, 160, 3). The recommended human dosage of Photofrin® is 1-2 mg/kg of body weight. The main components of Photofrin® are dimers and higher oligomers linked with ether, and possibly carbon-carbon linkages (Pandey, R.K.; Siegel, M.M.; Tsao, R.; McReynolds, J.M.; Dougherty, T.J., *Biomed. and Environ. Mass Spectrometry*, 1990, 19, 405).

For a photosensitizer to be clinically useful, it must be non-toxic, selectively taken up and/or retained in malignant tissues, activated by penetrating light (>600 nm), and photochemically efficient. Although Photofrin® has been approved for commercialization in Canada and is expected to be approved in other countries, including the United States, it lacks rapid clearance from tissues, is a complex mixture of oligomers, and has the disadvantage that its absorbance at 630 nm is not optimized for tissue penetration. New porphyrin photosensitizers are thus needed for the improvement of photodynamic therapy for cancer treatment.

Our search for more efficient, chemically pure, less phototoxic, and better localizing porphyrins was guided by patterns recognized in the variety of new porphyrins which have recently been shown to be successful PDT agents. The important porphyrin and chlorin derivatives which have led to the development of this research have been reviewed by Pandey, R.K.; Majchrzycki, D.F.; Smith, K.M.; Dougherty, T.J., *Proc. SPIE*, 1989, 1065, 104. The aspartyl derivatives of chlorin e_6, monoaspartyl chlorin e_6 and diaspartyl chlorin e_6, were found to be effective photosensitizers *in vitro* (Roberts,

Among long wavelength absorbing photosensitizers, bacteriochlorins have been proposed as potential useful candidates for use in photodynamic therapy (PDT) where strong absorptions in the visible spectrum can be used to photoactivate dyes previously located in targeted (neoplastic) tissues (Pandey, R.K.; Shiau, F.Y.; Isaac, M.; Ramaprasad, S.; Dougherty, T.J.; Smith, K.M., *Tetrahedron Lett.*, 1992, 33, 7815). Some naturally occurring bacteriochlorins, have previously been reported as effective photosensitizers both *in vitro* and as *in vivo* (Beems, E.M.; Dubbelman, T.M.A.R.; Lugtenburg, J.; Best, J.A.B.; Smeets, M.F.M.A.; Boehgeim, J.P.J., *Photochem. Photobiol.*, 1987, 46, 639). However, most of the naturally occurring bacteriochlorins (760-780 nm) are extremely sensitive to oxygen, which results in rapid oxidation to the chlorin state (640 nm); thus the spectroscopic properties of the bacteriochlorins are lost. Further, if a laser is used to excite the bacteriochlorin *in vivo*, oxidation may result in the formation of a new chromophore absorbing outside the laser window, thus reducing the photodynamic efficiency.

Brief Description of the Drawings

Figure 1 is a schematic equation showing the synthetic route to compounds 7 and 8.

Figure 2 is a schematic equation showing the synthetic route to compound 11.

Figure 3 is a schematic equation showing the synthetic route to compound 15.

Figure 4 is a schematic equation showing the synthetic route to compound 19.

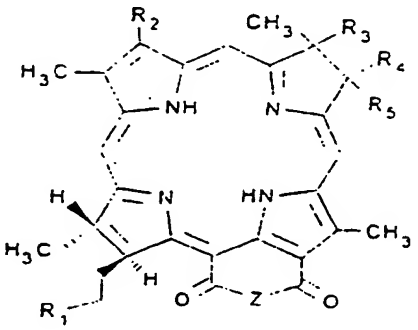
Figure 5 is a schematic equation showing the synthetic route to compounds 24 and 25.

Figure 6 is a schematic equation showing the synthetic route to compound 29.

Figures 7A through 7C show the structures of compounds 30, 31 and 32, respectively.

Figure 8 is a curve showing the light absorbance of compound 19.

In accordance with the invention, a compound is therefore provided which comprises a chemical of the formula:



where Z is = O or $=NR_6$, where R_6 is lower alkyl of from 1 to 8 carbon atoms or $-(CH_2)_4-\underset{NH_2}{CH}-CO_2Et$; R_1 is $-\overset{O}{\overset{\|}{C}}-R_7$, where R_7 is $-\underset{H}{N}R_8$ or $-OR_8$, where R_8 is hydrogen or lower alkyl of 1 to 8 carbon atoms, or R_7 is an amino acid residue connected at a nitrogen atom of such residue; R_2 is lower alkyl or lower alkylene of from 2 to 4 carbon atoms or a formal or carbonyl containing group of from 1 to 4 carbon atoms; R_3 and R_4 are -H or $-OR_8$ or are joined together so that each represents one-half of a chemical bond and R_4 may be taken together with R_5 to form =O; and R_5 is ethyl or is taken together with R_4 to form =O; provided that when Z is -O- the sum of the number of carbon atoms in R_1 through R_5 is from 12 to 20 and when Z is $=NR_6$, the sum of the number of carbon atoms in R_1 through R_6 is 8 to 20.

Detailed Description of the Invention

In order to prepare long wavelength absorbing photosensitizers, we modified purpurin-18, and two new bacteriochlorin systems, e.g., shown as structural formula 2, {in which a six membered anhydride ring is fused to the macrocycle} and e.g., 19, {in which the anhydride ring is replaced by an imide ring system}. Purpurin-18 methylester 2 was obtained from methyl pheophorbide-a 1 using a synthesis procedure known for other purposes (Kenner, G.W.; McCombie, S.W.; Smith, K.M., *J. Chem. Soc., Perkin Trans. I*, 1973, 2517). The anhydride ring in 2 was replaced with imide ring system 17 by first reacting purpurin-18 3 with lysine at room

products were isolated in 60 to 70% yield. As shown in Figs. 9 (for 11) and 8 (for 19) and in Tables A and B respectively, the formyl bacteriopurpurins 7, 8 and 10 and related imide derivative 15 and 19 have strong absorptions at 815 nm.

Table A - Compound 11

	PEAK		VALLEY	
NO.	nm	ABS	nm	ABS
1	813.0	0.337	623.0	0.046
2	602.0	0.052	600.0	0.046
3	546.0	0.231	490.0	0.054
4	404.0	0.328	381.0	0.233
5	360.0	0.518		

Table B - Compound 19

	PEAK		VALLEY	
NO.	nm	ABS	nm	ABS
1	816.0	0.386	806.0	0.040
2	602.0	0.045	600.0	0.039
3	546.0	0.242	488.0	0.052
4	406.0	0.360	383.0	0.296
5	361.0	0.605		

Some of the bacteriochlorins 7, 8, 10 and 19 were evaluated for *in vivo* photosensitizing efficacy. The preliminary results are summarized in Table 1. From these results it can be seen that formyl bacteriopurpurin-18 methylester 7 and aspartic acid di methyl ester 8 were found to be biologically inactive at a dose of 5.0 mg/kg, when treated 3h post i.v. injection of the drug. However, under similar treatment conditions, its aspartic acid di-tert butyl ester derivative 10 showed promising activity (80% tumor control, day 30). Bacteriochlorin 19, which still had propionic ester side chain at position 7, but wherein th anhydride ring has been replaced by imide ring system (R=L-lysine) showed much better activity (100% tumor control, day 7, 20% tumor control, day 30) than formyl bacteriopurpurin-18 7 and 8 (no tumor cure at all). Comparing the biological results of bacteriopurpurin 7 and 8 with 10, it is evident that aspartic acid substituents containing *tert*- butylester groups make significant difference in biological activity.

chemical shifts are reported relative to $CHCl_3$ at 7.258 ppm unless stated otherwise.

Analytical thin-layer chromatography was used to monitor reactions and to check purity of the desired compounds on cut strips (ca. 2 cm x 6 cm) of Merck or Whatman silica gel 60 F254 precoated (0.25 mm thickness) plastic backed sheets. Preparative TLC was performed on a freshly prepared glass plated (20 cm x 20 cm) coated with a ca 2 mm thick Merck silica gel CF254. Plates were activated prior to use by heating to 110°C for at least 8 hours. For column chromatography two types of packing was used: (i) Alumina was deactivated with 6% water (Brockman Grade III) before use; and (ii) Silica gel 60 (70-230 mesh) was used for normal gravity chromatography and silica gel 60 (230-400 mesh) was used for flash chromatography. Pressure for the later was supplied by house compressed air.

Before injecting the drug to animals the purity of the material was checked by HPLC, and it was performed using a Spectra-Physics system connected to SP8 700 solvent delivery system, Kratos 757 absorption detector with a fixed wavelength at 405 nm. Two solvent compositions were used in the HPLC analysis: (i) solvent A was prepared by dissolving anhydrous dibasic sodium phosphate (1.0 g) in 400 ml water. To this was added HPLC grade methanol (600 ml). The pH of the solution was adjusted to 7.5 with phosphoric acid; and (ii) solvent B was prepared by dissolving anhydrous dibasic sodium phosphate (0.3 g) in 100 ml water, and to this was added methanol (900 ml) and the pH was adjusted to 7.5 with phosphoric acid. For most of the photosensitizers (as methyl- or aspartylacid ditert-butylester derivatives) solvent B was used as isocratic mode (column, reverse phase C-8, flow rate 1.5 ml/min). For the intermediates as carboxylic acids, solvent A and solvent B were used as gradient mode (0.1-0 min A, 10-40 min A-B, 40-50 min B, 50-60 min back to A).

Tetrahydrofuran (THF) was distilled over sodium before use. All other solvents were used as commercially available (ACS grade). The phrase dried and filtered and evaporated means drying over sodium sulfate, filtering through glass

The reaction mixture was extracted with dichloromethane, washed with water till the pH of the aqueous phase in neutral. The organic phase was dried over anhydrous sodium sulfate, and the solvent was evaporated. The residue was crystallized from CH_2Cl_2/hexane as fine powder. Yield 80%. m.p. > 300°C. The NMR spectrum was same as discussed for the methyl ester except the resonances for the -OMe was missing.

Purpurin-18 asparticacid di-tert butylester 9: Purpurin-18 carboxylic acid 3 (200 mg) was dissolved in dry THF (100 ml). Dicyclohexylcarbodiimide (DCC, 250 mg), aspartic acid ditert butyl ester hydrochloride (200 mg), and dimethylaminioyridine (DMAP, 20 mg) was added to the reaction mixture. It was then stirred at room temperature for over night under nitrogen atmosphere. The reaction was monitored by analytical TLC. The reaction mixture was diluted with dichloromethane (200 ml), washed with water. The organic layer was separated, dried and evaporated. The residue was purified by preparative plates, using 5% methanol/dichloromethane. The major band was collected, washed with 5% methanol/dichloromethane till the silica is free from the title compound. Evaporation of the solvent and crystallization from dichloromethane/hexane gave the desired aspartyl derivative. Yield 160 mg. ^1H NMR (ppm): 9.30, 9.18, 8.54 (each H. 1H, meso H), 8.18 (dd, 1H, 2a-CH), 6.61 (d, 1H, Asp -NH), 6.27 (d, 1H, 2b-CH), 6.16 (d, 1H, 2b-CH cis to 2a-CH), 5.17 (d, 1H, 7-H), 4.67 (X Of ABX, 1H, Asp-CH), 4.31 (q, 1H, 8-H), 4.05 (q, 2H, 4a-CH_2), 3.59, 3.31, 3.03 (each s, 3H, CH_3), 3.58 (m, CH_2CH_2CO), 2.84, 2.73 and 2.48 (7b-CH, ABX 2H, Asp -CH_2), 1.72 (d, 3H, 8 CH_3), 1.58 (t, 3H, 4-CH_2CH_3). 1.39, 1.37 (each s, 9H, O-*tert*-butyl), 0.067 and -.194 (each br s, 1H, NH).

Purpurin-18 aspartic acid dimethylester 5: Purpurin-18 carboxylic acid (200 mg) was dissolved in dry THF (100 ml) and reacted with aspartic acid dimethyl ester (200 mg), DCC (250 mg), DMAP (20 mg) by following the method as discussed for the foregoing aspartic acid di tert butyl ester derivative, and the title compound was isolated in 80% yield.

Chlorin-p_6 6-lysylethoxyamide, 7-methyl ester 16, and Purpurin-N-lyselethoxi imide-7-methyl ester 17: To lysine ethyl ether, prepared by naturalization of lysine ethyl ester dihydrochloride (5 mg, 20 mmol) with aqueous KOH, purpurin-18 methyl ester (350 mg, 0.606) in chloroform (100 ml) was added. This mixture was stirred at room temperature under nitrogen overnight. The reaction was monitored by spectrophotometry (appearance of a peak at 665 nm, and disappearance of the peak of the starting material at 700 nm) and was worked up by following the standard methodology. The intermediate thus obtained was immediately dissolved in dichloromethane (100 ml) and montomorillonite K 10 (1 g) was added. The reaction mixture was stirred at room temperature under nitrogen, and was monitored by uv-vis spectroscopy (appearance of a new peak at 700 nm, and disappearance of the starting material peak at 665 nm). The reaction mixture was then filtered. Solvent was evaporated and the residue was purified by silica column, eluting with 2% methanol/dichloromethane. The title imide derivative was isolated in 60% yield starting from purpurin-18 methyl ester. m.p. 132-33°C. Analysis: Found: c, 68.7; H, 6.7; N; 11.3 $C_{42}H_{50}N_6O_6$ requires: C, 68.63; N, 6.86; N, 11.44%. max/nm 706 (), 664 (), 548 (), 510 (), 484 (), 418 (). ppm 9.56, 9.33, 8.59 (each s, meso H), 7.89 (dd, 2a-H), 6.29 (d, 2b-H), 6.16 (d, 2b'H), 5.40 (d, 7-H), 4.49 (m, 6-lysine CH_2), 4.36 (q, 8-H), 4.22 (q, 6-lysine $-CO_2CH_2$), 3.81 (s, 3H), 2.80-2.30 (m, 7-CH_2CH_2), 2.46 (m,-lysine-H), 1.79 (d, 8-Me), 1.66 (t, 4b-Me), 1.31 (t, 6-lysine-$CO_2CH_2CH_3$), -0.08 (br s, NH) and -0.17 (br s, NH) {Found m/z (HRMS) 734.3784. $C_{42}H_{50}N_6O_6$ requires 734.3792}.

Chlorin-p_6 6-N-hexylamide-7-methyl ester 12: A solution of purpurin-18 methyl ester (500 mg) in dichloromethane 950 ml) was cooled to 0°C and n-hexylamine (1.0 ml) was added to it. The reaction mixture was stirred at room temperature overnight under inert atmosphere, whereupon spectrophotometry and TLC showed the absence of starting material. The reaction mixture was then diluted with dichloromethane, washed with water. The dichloromethane layer was dried over anhydrous sodium sulfate,

diasteriomeric mixture) was isolated. ^1H NMR (ppm): [mixture of two isomers, *vic* diols are *cis*- up and *cis*- down relative to the ring D].

2-Formyl-*vic*-dihydr xy bacteriopurpurin-N-hexylimide-7-aspartic acid di-*tert*-butyl ester 24: As shown in Figure 5, for the preparation of the desired photosensitizer, the foregoing methyl ether analogue will be converted to corresponding carboxylic acid on reacting with aq. HCl. The carboxylic acid will be reacted with aspartic acid di-tert-butyl ester in presence of DCC and catalytic amount of DMAP, which on further reacting with osmium tetroxide/H_2S will giv the title bacteriopurpurin.

2-Formyl-*vic*-dihydroxy bacteriopurpurin-18, 7-methyl ester 7: 2-Formyl-purpurin-7-methyl ester 4 (mg) was dissolved in dichloromethane (ml), osmium tetroxide (mg) dissolved in diethyl ether (ml) and pyridine (ml) was added and the reaction mixture was stirred in a sealed flask (by using a rubber septum). The reaction was monitored by analytical TLC and spectrophotometry (appearance of a peak at 825 nm, and disappearance of the starting material peak at 734 nm). The osmate ester so obtained by converted to diol by bubbling a slow stream of H_2S gas through the solution. The reaction mixture was filtered, solvent was evaporated and the residue was purified by silica chromatography, eluting with 5% methanol in dichloromethane. The dark pink eluates were collected. After evaporating the solvent, the residue was crystallized from CH_2Cl_2/hexane. ^1H NMR (ppm), 11.40 (s, CHO), 9.70, 9.20, 8.10 (each s, meso H), 5.16 (m, 7-H), 4.35 (m, 8-H), 3.50-3.70 (2 and 5 ring CH_3 and CO_2CH_3), 3.62 (m, CH_2CH_2CO), 3.20, 2.70 (each m, 4-CH_2CH_3, and 7-bCH), 2.40 and 2.42 (each s, 3H, 3-CH_3), 1.76 (2 d, merged, 6H, 2 X 8 CH_3), -.10, -.18, -.30 and -.42 (s, NH protons).

2-Formyl-*vic*-dihydroxy bacteriopurpurin-18, 7-aspartic acid-di-tert-butyl ester 10: 2-Formyl purpurin-18 6 (mg) was reacted with osmium tetroxide () by following the methodology as discussed for the foregoing bacteriopurpurin and was

hydrophilicity/hydrophobicity plays an important role for an effective photosensitizer. Besides, those photosensitizers in which the hydrophilic groups are attached only at one half of the molecules were found to be more active than those in which these groups are present at both sides of the molecule. In order to achieve our objective, following two methods were used:

Method 1: In this approach, bacteriopurpurin **29** was obtained from bacteriochlorophyll-a **26**, which in turn can be isolated from *R. Spheroides* or *R. Capsulata*. In brief, bacteriochlorophyll-a **26** (5 mg) [obtained from the Porphyrin products, Logan, Utah, U.S.A.] was dissolved in diethylether (20 ml). 25% KOH (aqueous) in *i*- propanol was added and the reaction mixture was stirred at room temperature for 1h while the air was bubbled through it. Conc. HCl (5 ml) was added to the solution dropwise and the reaction mixture was stirred for another 0.5h. The reaction mixture was extracted with diethyl ether (3X50ml), washed with water till the pH of the aqueous phase is neutral. The ethereal layer was dried over anhydrous sulfate, and treated with ethereal diazomethane to convert the carboxylic acid to corresponding methylester. After evaporating the solvent, the residue was chromatographed on silica, eluting with chloroform-acetone as eluent. Evaporation of the solvent gave the desired bacteriopurpurin X in which the *vic*- dihydroxy groups are replaced with H substituents. Currently, efforts are being made to optimize the reaction conditions. max 361 (), 406 (), 540 (), 818 (). NMR ($CDCl_3$): 9.20, 8.80, 8.62 (each, meso H), 4.20 (7.8-H, m), 4.0 (3,4-H,m), 3.57, 3.47 (each s, 3H, CH_3), 3.15 (7b-H), 3.10 ($COCH_3$), 2.70 (7a-H, m), 2.26 (q, 2H, CH_2CH_3), 1.72(d,3H, 8-CH_3), 1.62 (d, 3H, 3-CH_3), 1.06 (m, 3H, CH_3), -0.38 (s, 1H, NH), -0.80 (s, 1H, NH).

For the preparation of aspartic acid derivatives **28**, efforts are currently being made to react the intermediate carboxylic acid to aspartic acid *di tert*- butyl ester by following the methodology as described for the preparation of other such derivatives. See Figure 6.

Experimental Procedure:

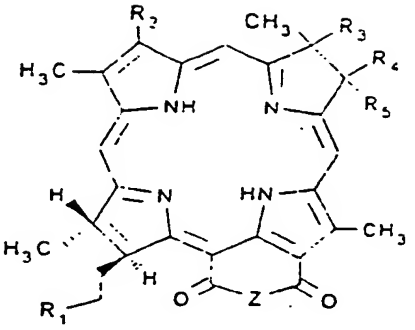
Following light exposure, the mice were kept in groups of 5 per cage and supplied with pelleted food and tap water ad libitum. Tumor size and gross appearance of both tumor and overlying surrounding skin was monitored daily for 80 days after photoillumination unless growth of non-responsive tumor require early sacrifice of those animals.

The photosensitizer was dissolved in known quantity of Tween-80 (Aldrich) surfactant and diluted by a factor of 10 with saline solution to produce a final Tween-80 concentration of 10%. The solution was then filtered through a syringe filter. The concentration of the solution was determined on the basis of the extinction coefficient value of the photosensitizer at the longest wavelength absorption. Absorption spectra were obtained using a Perkin Elmer 330 spectrophotometer.

Before injecting the drug into mice, the purity of the compounds was ascertained by analytical HPLC using Spectra Physics HPLC, connected with C8 reverse phase column, eluted with methanol/water by adjusting the pH to 7.0 using phosphate buffer.

WHAT IS CLAIMED IS:

1. A compound comprising:



where Z is = O or $=NR_6$, where R_6 is lower alkyl of from 1 to 8 carbon atoms or $-(CH_2)_4-\underset{NH_2}{CH}-CO_2$ Et; R_1 is $-\overset{O}{\overset{\|}{C}}-R_7$, where R_7 is $-\underset{H}{N}R_8$ or $-OR_8$, where R_8 is hydrogen or lower alkyl of 1 to 8 carbon atoms, or R_7 is an amino acid residue connected at a nitrogen atom of such residue; R_2 is lower alkyl or lower alkylene of from 2 to 4 carbon atoms or a formal or carbonyl containing group of from 1 to 4 carbon atoms; R_3 and R_4 are -H or $-OR_8$ or are joined together so that each represents one-half of a chemical bond and R_4 may be taken together with R_5 to form =O; and R_5 is ethyl or is taken together with R_4 to form =O; provided that when Z is -O- the sum of the number of carbon atoms in R_1 through R_5 is from 12 to 20 and when Z is $=NR_6$, the sum of the number of carbon atoms in R_1 through R_6 is 8 to 20.

2. The compound of Claim 1 wherein Z is $=NR_6$ and R_3 and R_4 are -OH.

3. The compound of Claim 2 wherein R_3 and R_4 are -OH.

4. The compound of Claim 2 wherein Z is $=NR_6$ and R_3 and R_4 are -H.

5. The compound of Claim 3 wherein R_7 is an amino acid residue.

FIG. 1

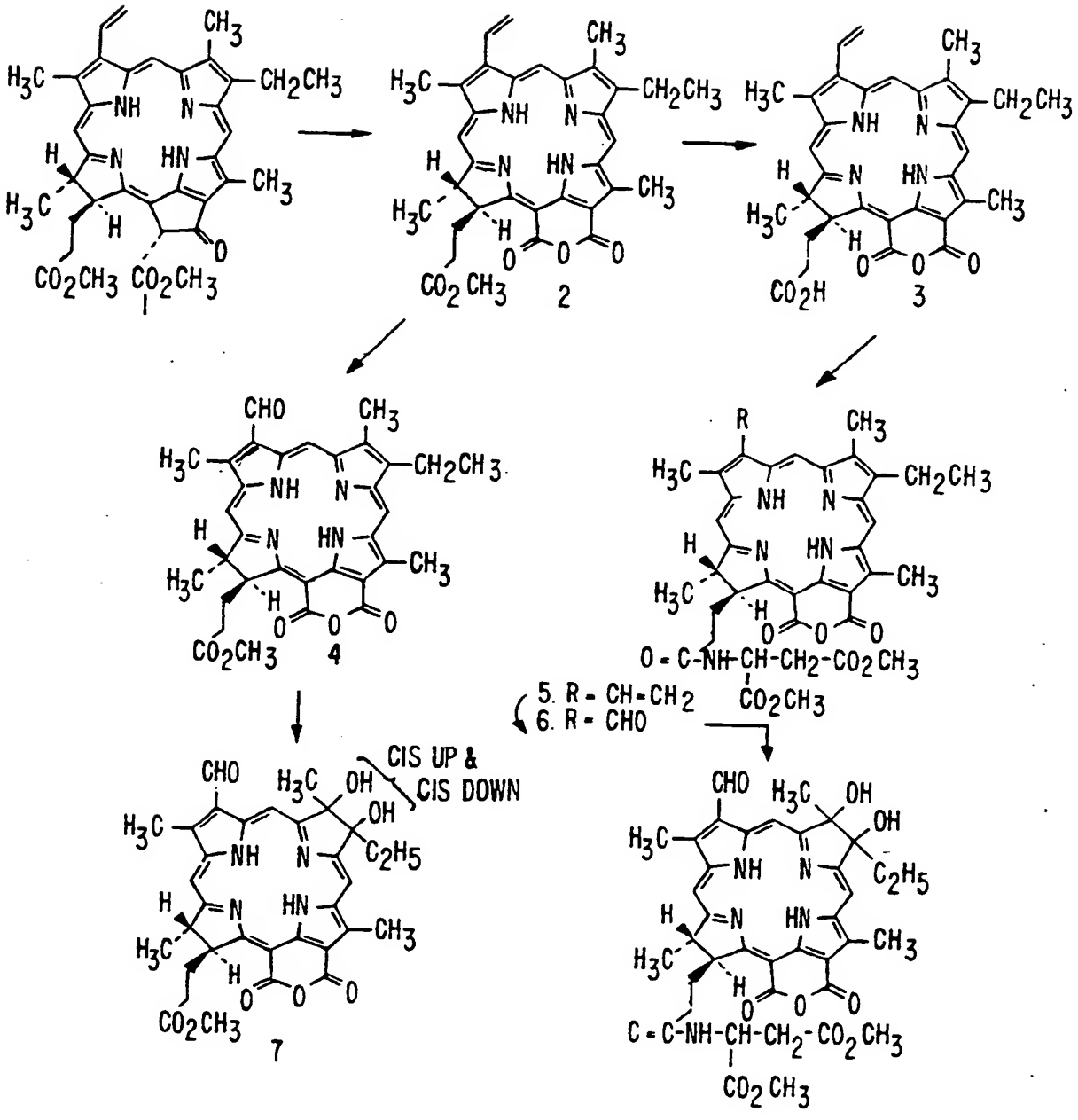
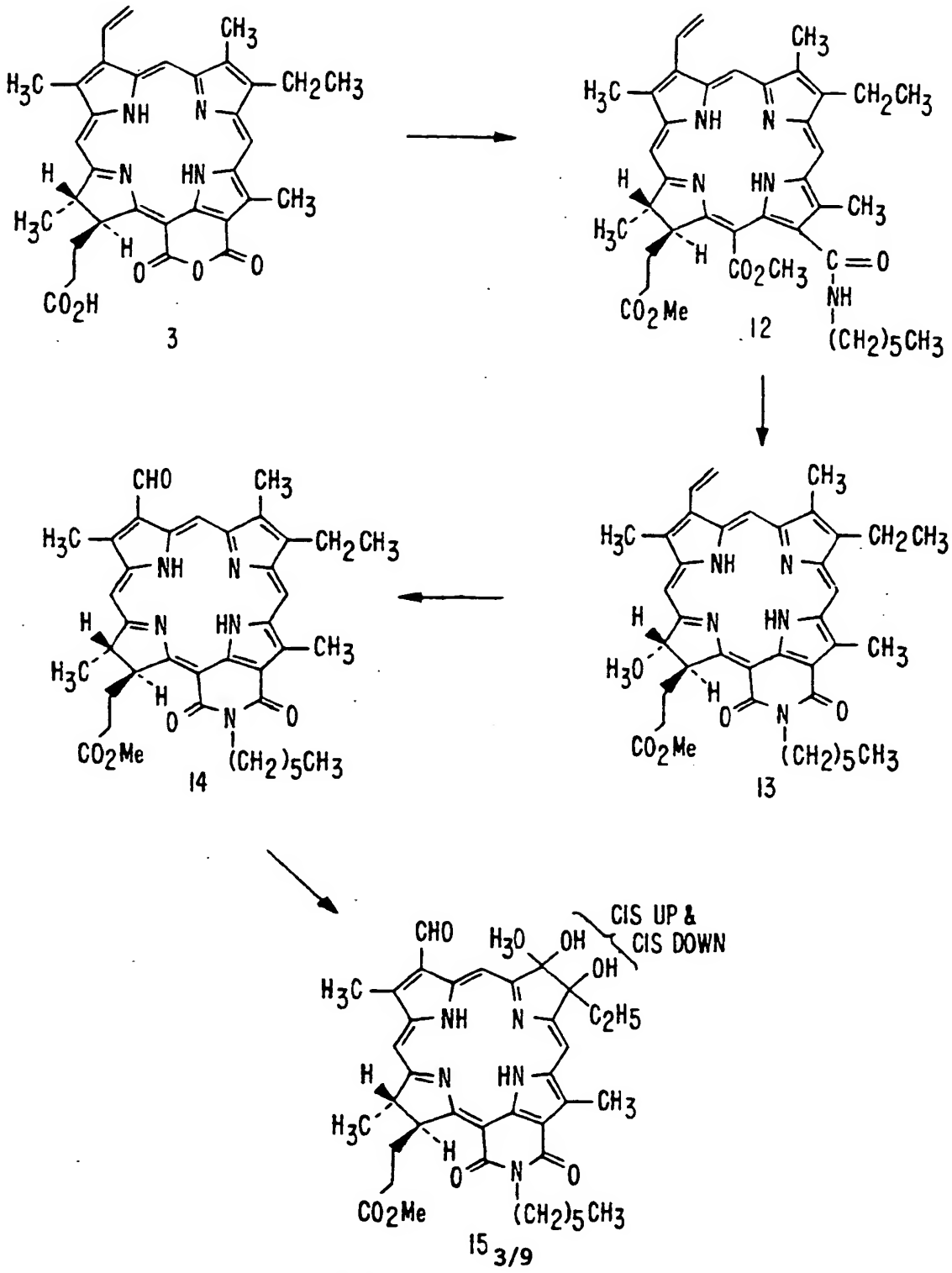


FIG. 3



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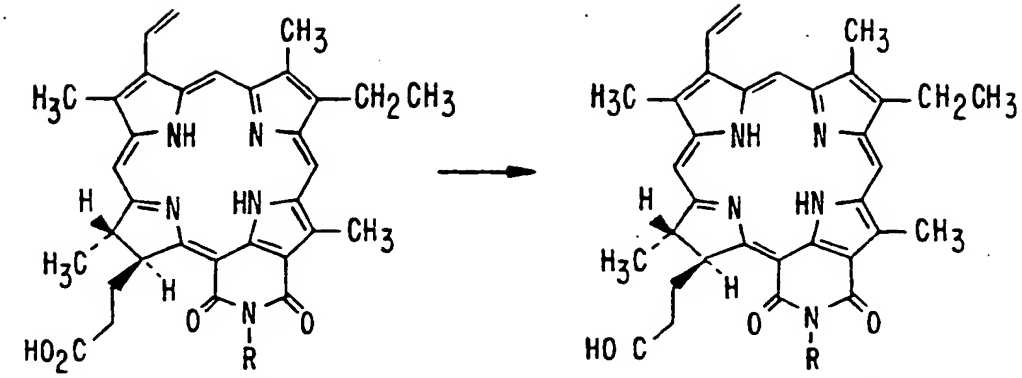
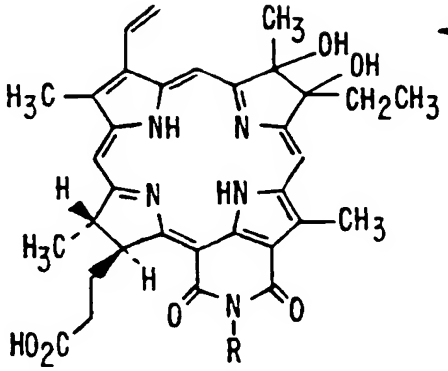
SUBSTITUTE SHEET (RULE 26)

FIG. 5



20. R = $(CH_2)_4$ $CH(CO_2Et)$ NH_2
21. R = $(CH_2)_5$ CH_3

22. R = $(CH_2)_4$ $CH(CO_2Et)NH_2$
23. R = $(CH_2)_5$ CH_3



24. R = $(CH_2)_4$ $CH(CO_2Et)NH_2$
25. R = $(CH_2)_5CH_3$

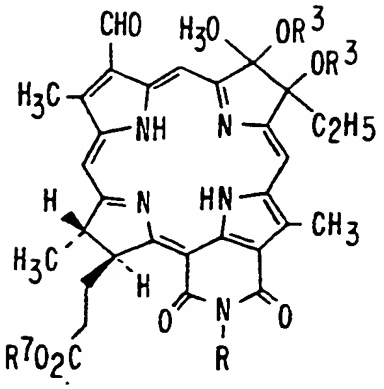


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FIG. 7A

R^7= methyl ester or aspartic acid di tert butyl ester
R^3= various alkyl groups
R = Various amino acids or alkyl groups

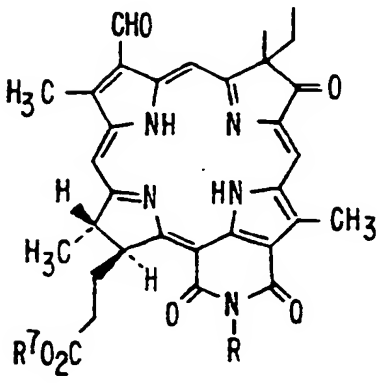

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FIG. 7B

R^7= methyl ester or aspartic acid di tert butyl ester
R^3= various alkyl groups
R = Various amino acids or alkyl groups

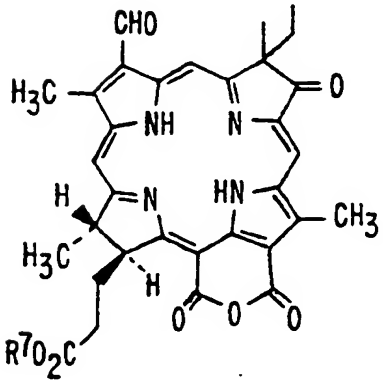

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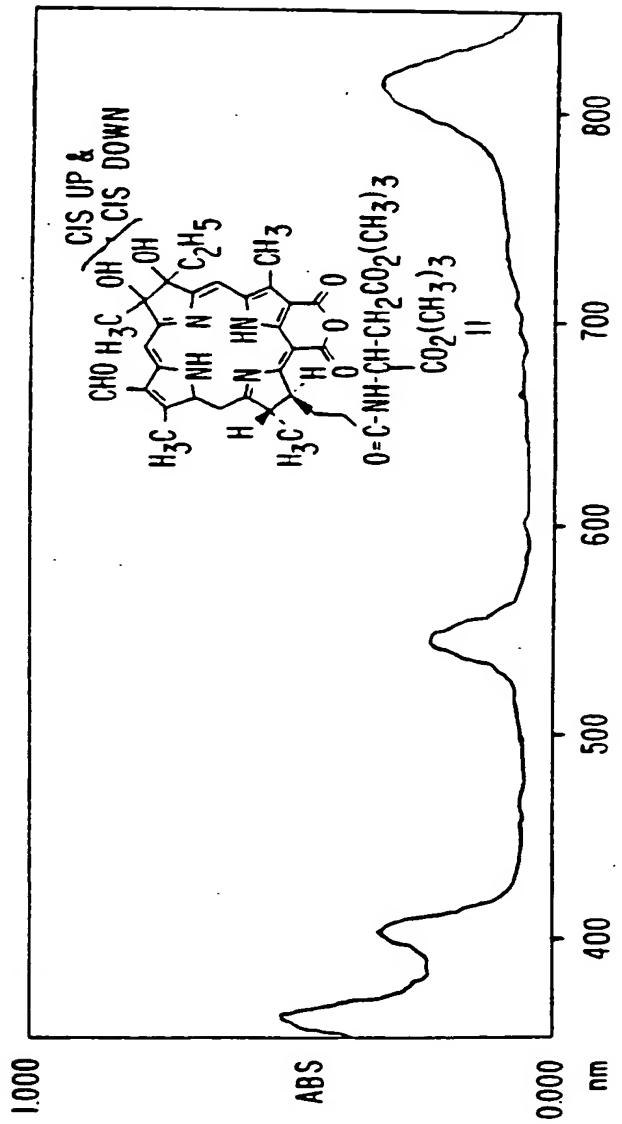
FIG. 7C

R^7= methyl ester or aspartic acid di tert buytl ester
R^3= various alkyl groups
R = Various amino acids or alkyl groups

FIG. 9



INTERNATIONAL SEARCH REPORT

International application No.
PCT/US95/06196

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	JOURNAL OF CHEMICAL SOCIETY, CHEMICAL COMMUNICATIONS, ISSUED AUGUST 1986, CHANG ET AL., "DIFFERENTIATION OF BACTERIOCHLORIN AND ISOBACTERIOCHLORIN FORMATION BY METALLATION. HIGH YIELD SYNTHESIS OF PORPHYRINDIONES VIA OSO OXIDATION', PAGES 1213-1215, SEE ENTIRE DOCUMENT.	1-19

FIG. 1

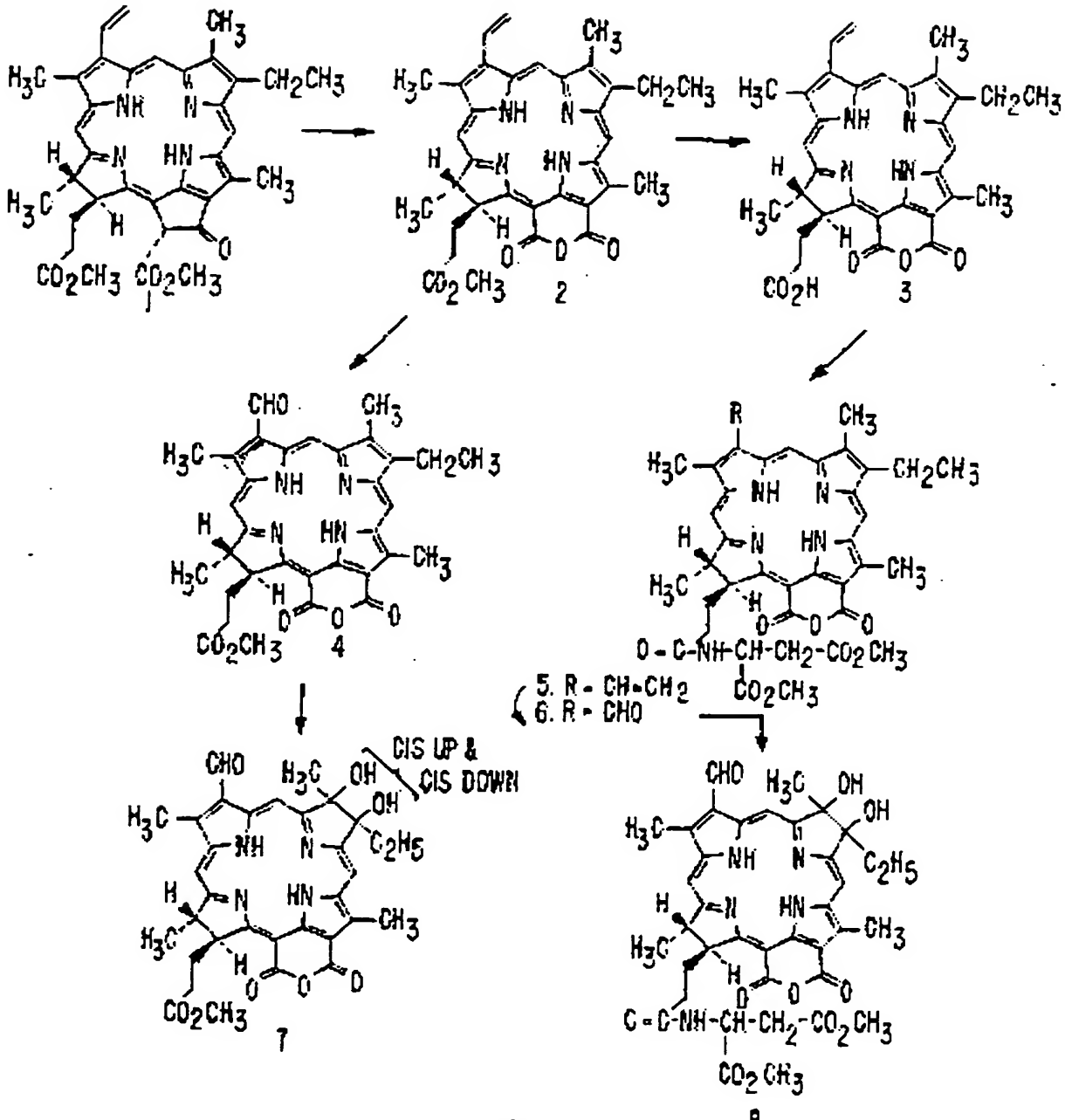
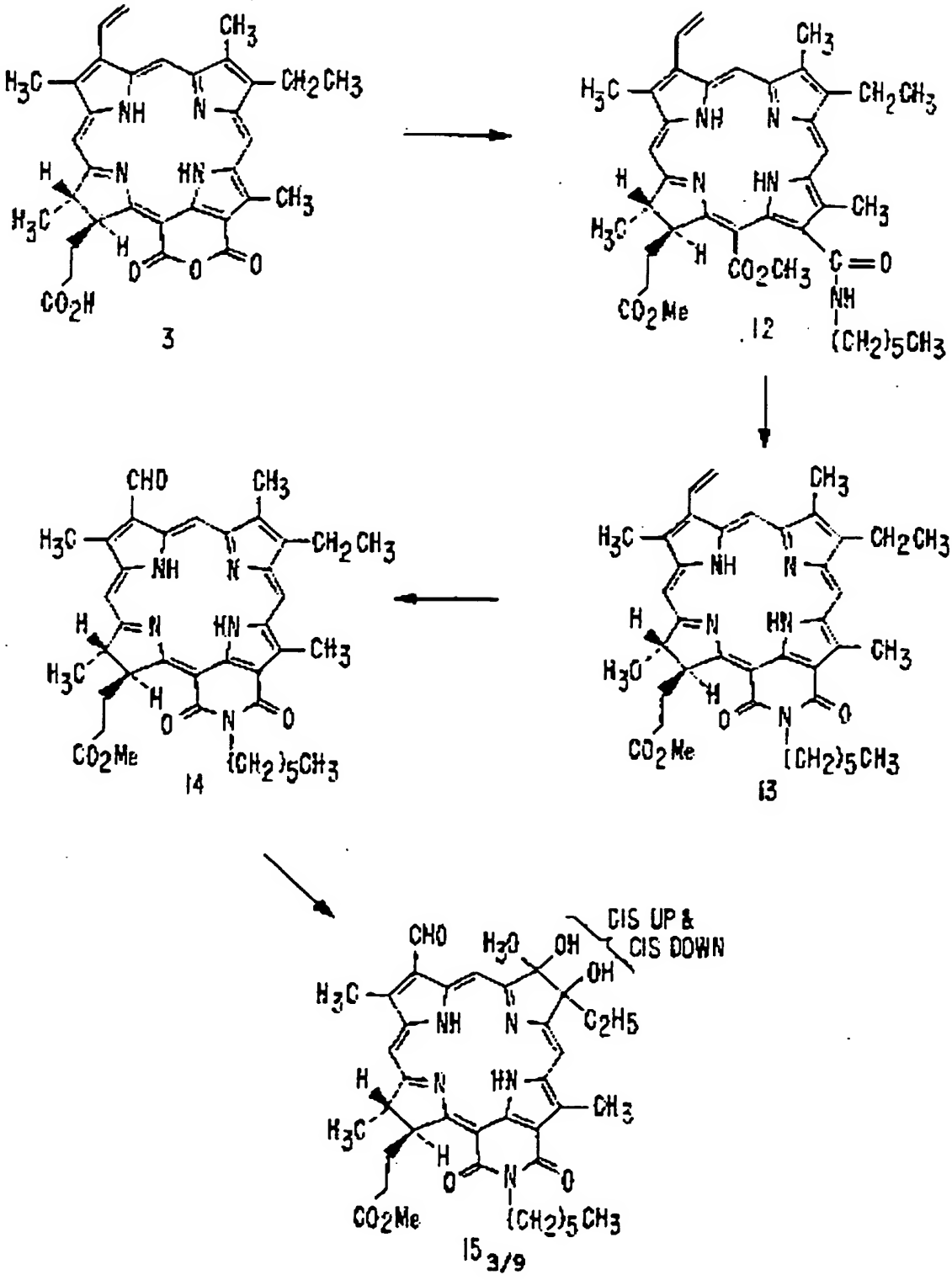
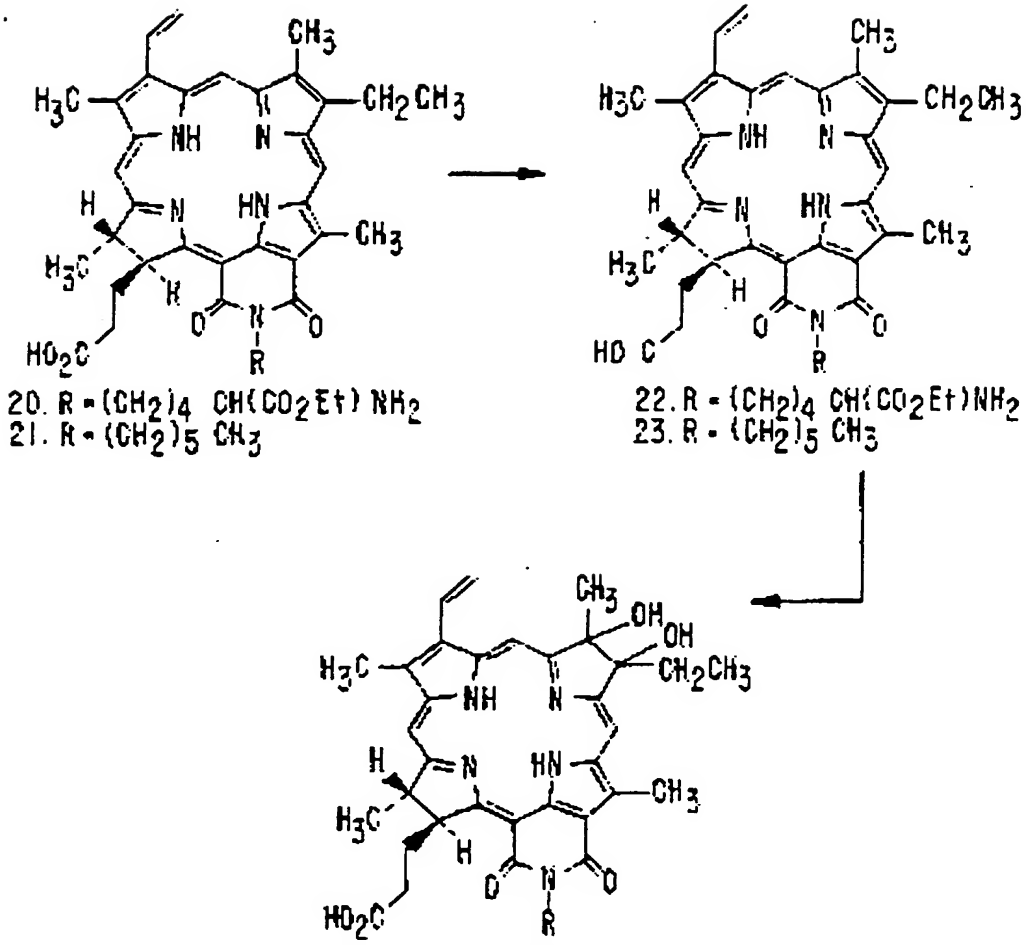


FIG. 3



SUBSTITUTE SHEET (RULE 26)

FIG. 5



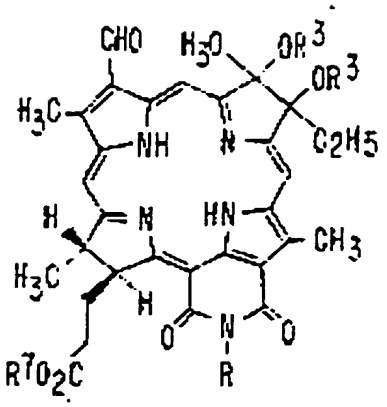


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FIG. 7A

R^7 - methyl ester or aspartic acid di tert butyl ester
R^3 - various alkyl groups
R - Various amino acids or alkyl groups

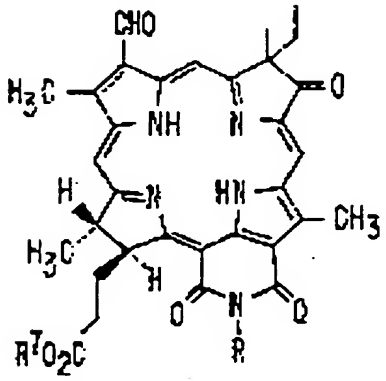

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FIG. 7B

R^7 - methyl ester or aspartic acid di tert butyl ester
R^3 - various alkyl groups
R - Various amino acids or alkyl groups

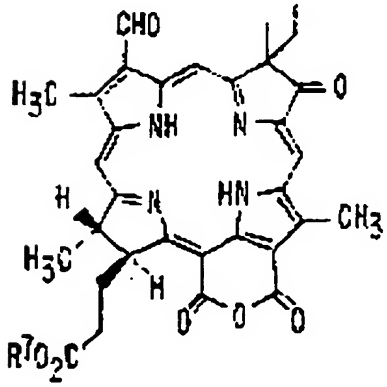

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FIG. 7C

R^7 - methyl ester or aspartic acid di tert buyti ester
R^3 - various alkyl groups
R - Various amino acids or alkyl groups

FIG. 9